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Modulation of Epinephrine-Stimulated Gluconeogenesis by Insulin in Hepatocytes Isolated from Genetically Obese *(fa/fa)* Zucker Rats*

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ABSTRACT

Genetically obese *(fa/fa)* Zucker rats present an impaired response of hepatic glucose production to the inhibition by insulin. In this work, we have investigated the modulation by this hormone of epinephrine-stimulated gluconeogenesis, in hepatocytes isolated from obese *(fa/fa)* rats and their lean *(Fa/-)* littermates. Epinephrine (1 μM) caused a maximal stimulation of [^{14}C]lactate conversion to [^{14}C]glucose in hepatocytes isolated from either obese or lean animals. The stimulation of gluconeogenesis by epinephrine was accompanied by a significant reduction of fructose 2,6-bisphosphate levels, an inactivation of both pyruvate kinase and 6-phosphofructo 2-kinase, and by a 2-fold increase in the cellular concentrations of cAMP. The presence of insulin in the incubation medium antagonized, in a concentration-dependent manner, the effects of epinephrine. In hepatocytes isolated from lean rats, the reversion caused by insulin was complete, the concentration required for half-maximal insulin action ranging from 0.22 to 0.56 nM. In contrast, in obese rat hepatocytes, insulin only partially blocked epinephrine-mediated effects, and the sensitivity to insulin was 2- to 4-fold lower, as indicated by the corresponding half-maximal insulin action values. Furthermore, insulin (10 nM) almost completely blocked the increase in cAMP levels induced by epinephrine in lean rat hepatocytes, whereas it only provoked a small and nonsignificant reduction of epinephrine-stimulated levels of the cyclic nucleotide in hepatocytes obtained from obese rats. (*Endocrinology* **138:** 2443–2448, 1997)

OBESITY IN the *(fa/fa)* Zucker rat is inherited as a recessive gene mutation, localized in chromosome 5 (1). Genetic mapping and genomic analysis have allowed the identification of *fa* as a mutation of the leptin receptor gene (2). The homozygous *(fa/fa)* rats show insulin resistance with hyperinsulinemia, hyperlipidemia, normal glycemia or mild hyperglycemia, and abnormal oral glucose tolerance (for reviews see Refs. 3–6).

In vitro (7–10) and *in vivo* (11, 12) studies have demonstrated that insulin resistance in genetically obese Zucker rats affects peripheral tissues, such as muscle and adipose tissue. The presence of insulin resistance at the hepatic level also was suggested by *in vivo* studies, in which it was observed that, as compared with lean *(Fa/-)* rats, the estimated hepatic glucose output in the obese animals was not reduced in spite of the presence of basal hyperinsulinemia (11); only after the infusion of very high concentrations of insulin was hepatic glucose production suppressed (12). More recently, we have demonstrated a decreased responsiveness to insulin of basal gluconeogenesis from lactate, and of F-2,6-P_2 levels, as well as of pyruvate kinase and 6-phosphofructo 2-kinase (PFK-2) activities, in hepatocytes isolated from obese *(fa/fa)* rats, as compared with that observed in lean rat hepatocytes (13).

In this work, we have studied the modulation by insulin of epinephrine-stimulated gluconeogenesis, in hepatocytes isolated from obese *(fa/fa)* Zucker rats and their lean *(Fa/-)* littermates. Our results show that, in hepatocytes isolated from lean *(Fa/-)* rats, insulin (in a concentration-dependent manner) completely antagonized the stimulation of lactate gluconeogenesis elicited by a saturating concentration of epinephrine (1 μM), as well as the effects of this adrenergic agent on F-2,6-P_2 levels and pyruvate kinase and PFK-2 activities. In contrast, insulin only partially blocked the metabolic actions of epinephrine in hepatocytes isolated from obese *(fa/fa)* rats, these cells being less sensitive to modulation by insulin. Moreover, the inhibition by insulin (10 nM) of the increase in cAMP levels elicited by epinephrine also was impaired in hepatocytes obtained from obese rats.

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Materials and Methods

Reagents

Human insulin (Actrapid HM) was obtained from Novo Industri A/S (Copenhagen, Denmark). Collagenase A, F-2,6-P_2, substrates, auxiliary enzymes, and coenzymes were purchased from Boehringer Mannheim (Mannheim, Germany). Epinephrine [(+)-bitartrate salt] was obtained from Sigma (St. Louis, MO). L-[U-^{14}C]lactate (150 μCi/μmol) was provided by Amersham International (Aylesbury, UK). The remaining reagents, all of analytical grade, were from Boehringer, Sigma, or Merck (Darmstadt, Germany).

Animals

Male lean *(Fa/-)* and genetically obese *(fa/fa)* rats weighing 300–360 and 430–500 g, respectively, were obtained from Criffa (Barcelona, Spain). The rats were 14–17 weeks old at the time of experiments. They were fed with a standard chow (A 04 Panlab S.L., Barcelona, Spain) and water *ad libitum*, and were housed in animal quarters at constant temperature (23 C) with a fixed (12-h) light cycle.

Hepatocyte isolation and cell incubations

Hepatocytes were isolated by perfusion of the liver with collagenase (14). Cells were suspended in Krebs-Henseleit medium (40–60 mg wet wt/ml) in the presence of 10 mM glucose and incubated in stoppered 20-ml vials, at 37 C, with agitation (100 strokes/min). The gas phase was 95% O_2 and 5% CO_2. The viability of the isolated hepatocytes was evaluated by the trypan blue test; usually 90–95% of the cells excluded the stain.

Gluconeogenesis was estimated by the rate of [U-^{14}C]lactate conversion to [^{14}C]glucose, from a mixture of [U-^{14}C]lactate/pyruvate (2/0.2 mM; 0.5 μCi/μmol) (15). Hepatocytes were preincubated in Krebs-Henseleit medium in the presence of 10 mM glucose for 27 min. Then, saline or insulin was added to the hepatocyte suspensions, and the preincubation further continued. Three minutes later, saline or epinephrine, together with the gluconeogenic precursor, were added to the cell suspensions (zero time of incubation). At the indicated times, aliquots of cell suspensions were taken to assay gluconeogenesis, F-2,6-P_2, cAMP, or enzyme activities. The actual specific activity of [^{14}C]lactate was assayed in aliquots of cell suspensions taken at zero time.

Biochemical procedures

As previously described (15), hepatocyte F-2,6-P_2 was measured in aliquots of cell suspensions, taken after 10 min incubation, by the ability of this metabolite to activate potato tuber PPi:fructose 6-phosphate 1-phosphotransferase (16). For the assay of cAMP, aliquots of cell suspensions were taken after ten min incubation; the cyclic nucleotide was determined in cells plus medium using a radioimmunological method (cAMP (^{125}I)RIA Kit, Du Pont de Nemours, Bad Homburg, Germany), as described elsewhere (14). Reported methods were used to assay pyruvate kinase activity (17) and the active form of PFK-2 (18). Pyruvate kinase was measured in the presence of 0.15 mM phosphoenolpyruvate. Protein was assayed by the method of Lowry *et al.* (19) using BSA as standard; 1 g of packed hepatocytes corresponded to 220 ± 5 mg of protein.

Statistical analysis

Statistical significance of differences between values was calculated by the paired and unpaired Student's *t* test. The differences were considered statistically significant when *P*-value was less than 0.05. The concentrations of insulin corresponding to the half-maximal effects (EC_{50}) were calculated by the nonlinear equation fitting utility of the graphics program Fig.P (Fig.P Software Corporation, Durham, NC).

Results

Basal gluconeogenesis from lactate was significantly reduced in hepatocytes isolated from obese *(fa/fa)* rats, as compared with that measured in lean *(Fa/-)* rat hepatocytes (0.50 ± 0.06 and 1.96 ± 0.15 μmol of lactate converted to glucose/g cell × 20 min, respectively; n = 3; $P < 0.01$). These results are in agreement with the reduction of lactate gluconeogenesis observed by Bloxham and York (20) and by our own group (13) in hepatocytes isolated from genetically obese rats. To select a saturating concentration of epinephrine, we assayed the influence of increasing concentrations of this catecholamine on hepatocyte gluconeogenesis from a mixture of [^{14}C]lactate-pyruvate, in liver cells isolated from either obese or lean animals. As shown in Fig. 1, incubation with epinephrine caused a dose-dependent stimulation of

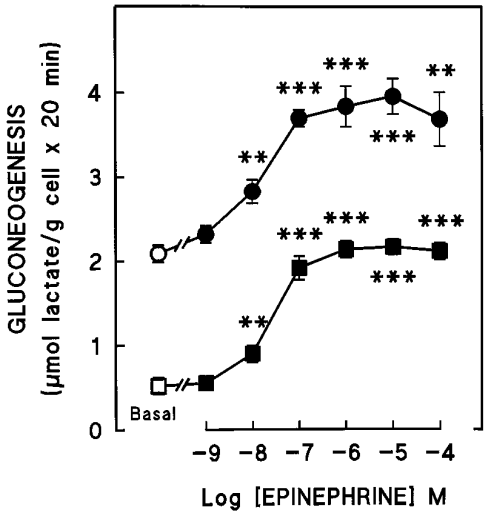

FIG. 1. Dose-response curve of the epinephrine effect on basal gluconeogenesis in hepatocytes isolated from lean (○,●) or obese (□,■) Zucker rats. Hepatocytes were suspended in Krebs-Henseleit medium in the presence of 10 mM glucose and preincubated for 30 min at 37 C. Then, a mixture of [U-^{14}C]lactate-pyruvate together with saline or epinephrine at the indicated concentrations was added, and the incubation continued. Gluconeogenesis was assayed in aliquots of the cell suspensions taken after 20 min of incubation. Results are expressed as mean ± SEM of four observations from two experiments. By unpaired Student's *t* test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ (*vs.* basal incubations). Basal values were obtained in hepatocytes incubated in the absence of hormones.

[^{14}C]lactate conversion to [^{14}C]glucose; a maximal stimulation of hepatocyte gluconeogenesis was already achieved in the presence of 1 μM epinephrine in both types of cells. The calculated EC_{50} values of epinephrine were 26.9 and 14.7 nM, respectively, for obese and lean rat hepatocytes. It is of note that although the maximal rate of epinephrine-stimulated gluconeogenesis was much higher in lean rat hepatocytes than in hepatocytes isolated from obese animals, the response to saturating concentrations of epinephrine actually was more marked in obese rat liver cells than in lean rat hepatocytes (about 4- and 1.8-fold, respectively, as compared with the corresponding basal gluconeogenesis). However, when the areas under the curves for obese and for lean rat liver cells were calculated, the increments over the corresponding basal rate of gluconeogenesis caused by epinephrine were not significantly different (respectively, 5.98 ± 0.46 and 6.94 ± 0.37; values are expressed in arbitrary units; $P > 0.05$; n = 4 observations from two experiments). This indicates that the stimulation of gluconeogenesis from lactate caused by epinephrine was quantitatively similar in obese and lean rat hepatocytes.

In hepatocytes incubated with 1 μM epinephrine, the additional presence of insulin in the incubation medium reduced epinephrine-stimulated gluconeogenesis in a concentration-dependent manner (Fig. 2). In hepatocytes isolated from lean rats, a complete reversion of the stimulation of gluconeogenesis caused by epinephrine was observed in the presence of 0.1 μM insulin, 0.35 nM being the calculated EC_{50} value. In contrast, in obese rat hepatocytes, the reversion of the epinephrine effect was not complete ($P < 0.05$, for 0.1 μM insulin *vs.* the corresponding basal value in the absence of

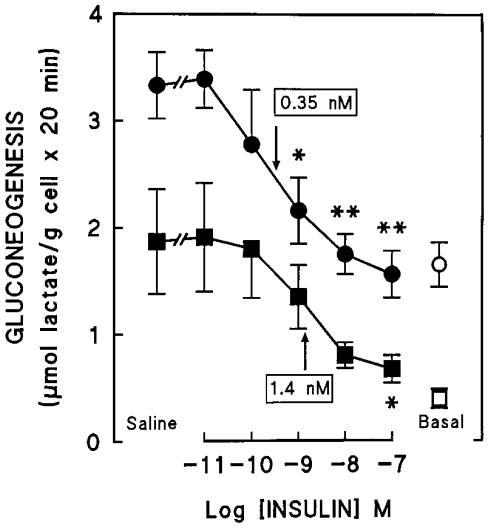

FIG. 2. Dose-response curve of the insulin effect on epinephrine-stimulated gluconeogenesis in hepatocytes isolated from lean (○,●) or obese (□,■) Zucker rats. Hepatocytes were suspended in Krebs-Henseleit medium in the presence of 10 mM glucose and preincubated for 27 min at 37 C. Then, saline or insulin at the indicated concentrations was added to the incubation medium. Three minutes later, a mixture of [U-^{14}C]lactate-pyruvate together with epinephrine (1 μM) was added, and the incubation continued. Gluconeogenesis was assayed in aliquots of the cell suspensions taken after 20 min of incubation. Results are expressed as mean ± SEM of four experiments. By paired Student's *t* test: *, $P < 0.05$; **, $P < 0.01$ (*vs.* saline incubations). Basal values were obtained in hepatocytes incubated in the absence of hormones. EC_{50} values for insulin are indicated in *boxes*.

hormones), and the apparent sensitivity to insulin was lower than that observed in lean rat hepatocytes, with a calculated EC_{50} value of 1.4 nM.

The action of insulin on the activities of pyruvate kinase and PFK-2, enzymes which play a key role in the short-term hormonal control of gluconeogenesis (21, 22), also was evaluated in liver cells incubated with epinephrine. According to previous reports (13, 20, 23–25), obese rat hepatocytes showed higher basal levels of pyruvate kinase activity (0.15 mM phosphoenolpyruvate) than those isolated from lean rats (61.9 ± 2.6 and 26.4 ± 1.0 U/g of cells, respectively; n = 4; $P < 0.001$). As expected, epinephrine caused a marked inactivation of pyruvate kinase (about 60%) in liver cells isolated from both animal groups (Fig. 3A). The presence of insulin antagonized, in a dose-dependent manner, the effects of epinephrine on this enzymatic activity. In lean rat hepatocytes, the reversion caused by insulin was complete, and the calculated EC_{50} value was 0.56 nM. On the contrary, in hepatocytes isolated from obese rats, insulin (even at saturating concentrations) only partially antagonized epinephrine-mediated pyruvate kinase inactivation ($P < 0.05$, for 0.1 μM insulin *vs.* the corresponding basal value in the absence of hormones). Furthermore, these cells showed a lower sensitivity to insulin than that observed in lean rat hepatocytes, with an EC_{50} value of 1.6 nM (Fig. 3A).

In good agreement with other reports (13, 26), PFK-2 *a* activity was significantly higher in liver cells isolated from obese rats than in those obtained from lean animals (7.33 ± 0.35 and 6.07 ± 0.27 mU/g of cells, respectively; n = 4; $P < 0.05$). As shown in Fig. 3B, epinephrine also caused the inactivation of PFK-2 in both lean and obese rat hepatocytes (about 50% and 60% inactivation, respectively). The additional presence of insulin in the incubation medium antagonized, in a concentration-dependent fashion, the effects of epinephrine in hepatocytes isolated from either obese or lean rats. In the latter cells, the reactivation of PFK-2 caused by 0.1 μM insulin led to a value even higher than the basal level estimated in the absence of hormones ($P < 0.05$). On the contrary, in obese rat hepatocytes, insulin did not antagonize the epinephrine-mediated inactivation of PFK-2 completely ($P < 0.05$, for 0.1 μM insulin *vs.* the corresponding basal value), and the apparent sensitivity to insulin was lower than that observed in lean rat cells (EC_{50} values of 0.22 and 0.89 nM for hepatocytes isolated from lean and obese animals, respectively).

Considering that F-2,6-P_2 is a key regulatory metabolite in the hormonal control of hepatic gluconeogenesis (21, 22), we also have studied the modulation by epinephrine and insulin of the content of this metabolite in hepatocytes isolated from obese *(fa/fa)* rats and their lean littermates. As previously reported (13, 25, 26), obese rat hepatocytes showed a marked

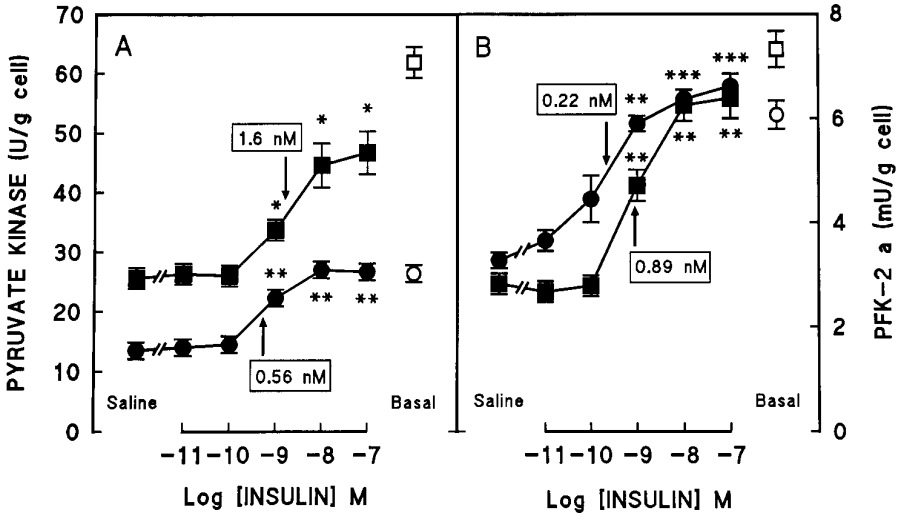

FIG. 3. Dose-response curve of the insulin effects on pyruvate kinase (panel A) and PFK-2 (panel B) activities in epinephrine-incubated hepatocytes from lean (○,●) or obese (□,■) Zucker rats. Additions to the cell suspensions were as in Fig. 2. Enzyme activities were measured in aliquots of the cell suspensions taken after 10 min of incubation. Results are expressed as mean ± SEM of four experiments. By paired Student's *t* test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (*vs.* saline incubations). Basal values were obtained in hepatocytes incubated in the absence of hormones. EC_{50} values for insulin are indicated in *boxes*.

elevation of F-2,6-P_2 levels, as compared with those observed in lean rat hepatocytes (14.5 ± 0.8 and 6.0 ± 0.6 nmol/g of cells, respectively; n = 4; $P < 0.001$). Epinephrine provoked a significant reduction in the F-2,6-P_2 content in hepatocytes isolated from either obese or lean animals (by about 60% and 40%, respectively) (Fig. 4). The additional presence of insulin in the incubation medium caused a clear increase in the levels of this metabolite in liver cells from both animal groups; furthermore, in lean rat hepatocytes, F-2,6-P_2 eventually reached values significantly higher than those measured in the absence of hormones ($P < 0.01$). Again, the sensitivity to insulin seemed to be lower in obese rat hepatocytes than in cells isolated from lean rats, the EC_{50} values for insulin corresponding to 0.71 and 0.35 nM, respectively (Fig. 4).

To elucidate whether the differences found between obese and lean rat hepatocytes in the ability of insulin to antagonize the actions of epinephrine on gluconeogenesis (as well as on the other metabolic parameters studied) could be caused by changes in the modulation of the cellular concentrations of cAMP, the levels of this second messenger were measured. As shown in Table 1, the basal content of cAMP was similar in obese and lean rat hepatocytes; the addition of 1 μM epinephrine to the incubation medium raised the levels of the cyclic nucleotide about 2-fold in both types of cells. In lean rat hepatocytes, the additional presence of insulin (10 nM) reduced the increase in cAMP content caused by epinephrine by about 80%. In contrast, in hepatocytes isolated from obese rats, the inhibitory effect of insulin on epinephrine-stimulated cAMP levels was not statistically significant.

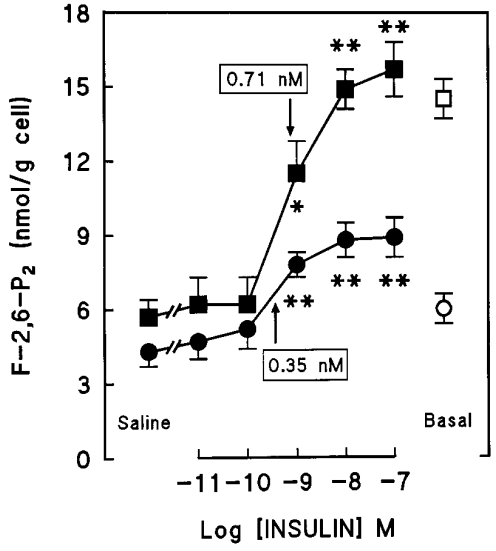

FIG. 4. Dose-response curve of the insulin effect on F-2,6-P_2 levels in epinephrine-incubated hepatocytes from lean (○,●) or obese (□,■) Zucker rats. Additions to the cell suspensions were as in Figure 2. F-2,6-P_2 concentrations were assayed in aliquots of the cell suspensions taken after 10 min of incubation. Results are expressed as mean ± SEM of four experiments. By paired Student's *t* test: *, $P < 0.05$; **, $P < 0.01$ (*vs.* saline incubations). Basal values were obtained in hepatocytes incubated in the absence of hormones. EC_{50} values for insulin are indicated in *boxes*.

TABLE 1. Effects of epinephrine and insulin on the cellular levels of cAMP in hepatocytes isolated from obese (*fa/fa*) and lean (*Fa/−*) rats

	Basal	Epinephrine (1 μM)	Epinephrine + insulin (1 μM/10 nM)
Lean rats	0.25 ± 0.02	0.55 ± 0.07[a]	0.31 ± 0.02[b,c]
Obese rats	0.25 ± 0.03	0.49 ± 0.02[a]	0.43 ± 0.03[d,c]

Hepatocytes were suspended in Krebs-Henseleit medium in the presence of 10 mM glucose and preincubated for 27 min at 37 C. Then, saline or insulin were added to the incubation medium. Three minutes later, a mixture of lactate-pyruvate together with saline or epinephrine was added to the incubation medium. After 10 min of incubation, aliquots of the cell suspensions were taken for cAMP assay. Values are the mean ± SEM of three experiments assayed in duplicate and are expressed as nmol per g of cells. Significance was determined by paired Student's *t* test.

[a] $P < 0.05$, epinephrine *vs.* saline incubations.
[b] $P < 0.05$, epinephrine plus insulin *vs.* epinephrine incubations.
[c] $P < 0.05$, epinephrine plus insulin *vs.* saline incubations.
[d] P = NS, epinephrine plus insulin vs. epinephrine incubations.

Discussion

The mechanisms underlying short-term modulation of hepatic gluconeogenesis by adrenergic agonists and insulin are well documented (reviewed in Refs. 21 and 22). In rat hepatocytes incubated under physiological conditions, epinephrine has been shown to stimulate gluconeogenesis (17, 27) through a β-adrenergic receptor-dependent stimulation of adenylate cyclase and the consequent increase in cAMP levels (27). The increase in the concentration of this second messenger causes the activation of protein kinase A, which in turn, phosphorylates and inactivates pyruvate kinase (17). Protein kinse A also phosphorylates the key enzyme 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase (PFK-2/FBPase-2), causing the inactivation of PFK-2 (18, 28) and the activation of FBPase-2 (18, 28). The diminution in the PFK-2/FBPase-2 activity ratio provokes a decrease in the hepatocyte F-2,6-P_2 content. As a consequence of these changes, epinephrine increases the gluconeogenic flux (22). On the other hand, insulin is able to antagonize all these epinephrine-mediated metabolic changes in a coordinated fashion (17, 27, 28) through the inhibition of the cellular cAMP increase caused by the catecholamine (22, 27).

According to these concepts, in hepatocytes isolated from lean *(Fa/-)* and obese *(fa/fa)* rats, epinephrine caused a marked stimulation of lactate gluconeogenesis, a simultaneous inactivation of both pyruvate kinase and PFK-2 activities, and a marked decrease in F-2,6-P_2 levels. In hepatocytes isolated from lean rats, insulin completely antagonized the stimulation of lactate gluconeogenesis elicited by epinephrine (1 μM), as well as the effects of this adrenergic agent on F-2,6-P_2 levels and on pyruvate kinase and PFK-2 activities. In contrast, in obese rat hepatocytes, insulin only partially blocked epinephrine effects on gluconeogenesis and pyruvate kinase and PFK-2 activities and caused a lower increase in F-2,6-P_2 levels than that observed in hepatocytes from lean animals. These results support the concept of a decreased response of epinephrine-stimulated gluconeogenesis to insulin counteraction in obese *(fa/fa)* rat hepatocytes. Furthermore, in these cells, the corresponding EC_{50} values for the action of insulin on the assayed metabolic parameters were 2- to 4-fold higher than those estimated for liver cells isolated from lean rats,

indicating the presence of a reduced sensitivity to insulin in obese rat hepatocytes.

Insulin resistance in the obese *(fa/fa)* rats affects liver, muscle, and adipose tissue (3–6). At the hepatic level, these animals have been shown to display a reduced sensitivity to the inhibition by insulin of hepatic glucose production (11, 12). In hepatocytes isolated from obese *(fa/fa)* rats, an impaired response to insulin was evidenced in both apo-B lipoprotein (29, 30) and triacylglycerol secretion (30). Although the primary cause of hepatic insulin resistance in obese *(fa/fa)* rats is not fully understood, current evidence indicates that both receptor and postreceptor events are affected, because decreases in hepatic insulin binding (31) and insulin receptor tyrosine kinase activity (32) have been reported in these animals. We also found an impairment of a glycosyl-phosphatidylinositol-dependent insulin signaling system in hepatocytes isolated from obese *(fa/fa)* rats (33). On the other hand, it has been reported that these cells present a defective function of the inhibitory guanine-nucleotide-binding protein G_i-2 (34), probably caused by an abnormal hyperphosphorylation of αG_i-2 subunit (35). Recently, it has been demonstrated that αG_i-2 deficiency in mice provokes an impairment in the actions of insulin in liver, skeletal muscle, and adipose tissue (36).

With regard to the modulation by insulin of epinephrine-mediated effects in the liver, we have found no previous data concerning this hormonal regulation in obese *(fa/fa)* rats. In good agreement with our findings, Marette *et al.* (37) found similar defects in both sensitivity and responsiveness to insulin in brown adipocytes isolated from obese *(fa/fa)* rats, when the inhibitory actions of insulin on norepinephrine-stimulated oxygen consumption and lipolysis were studied.

As mentioned earlier, the mechanism by which insulin antagonizes the stimulation of gluconeogenesis by epinephrine in isolated rat hepatocytes seems to involve an inhibition of the cellular cAMP increase caused by the catecholamine (22, 27). Accordingly, in hepatocytes isolated from lean *(Fa/-)* rats, insulin caused an 80% reduction of the 2-fold increase in cAMP levels elicited by epinephrine. In contrast, insulin (at this concentration) was unable to reduce significantly the cellular content of this second messenger in obese rat hepatocytes incubated with epinephrine. This suggests that in hepatocytes from obese rats, the observed resistance to the antagonistic effect of insulin on the stimulation of gluconeogenesis by epinephrine could be related to a defect in the modulation by insulin of the epinephrine-mediated increase in cAMP levels. As mentioned above, hepatocytes isolated from these animals show a defect in G_i protein function (34, 35), and this abnormality could be responsible, at least in part, for the impairment of insulin-mediated reduction of cAMP levels observed in this animal model of insulin resistance.

It must be mentioned that there is some evidence suggesting that either phenylephrine or epinephrine, apparently acting through their binding to α_1-adrenergic receptors, may stimulate hepatic gluconeogenesis by a cAMP-independent mechanism (27, 38–40). Insulin is able to antagonize this stimulation, in the absence of changes in either the cellular content of the nucleotide or the activity of protein kinase A (27, 40). In this regard, it has been suggested that under basal conditions, insulin could activate both hepatic PFK-2 and pyruvate kinase by the stimulation of protein phosphatases (41, 42); it also has been reported that insulin causes a transient stimulation of a Mg^{2+}-dependent pyruvate kinase phosphatase activity in isolated rat hepatocytes (43). The operation of this cAMP-independent mechanism of insulin action could explain the fact that this hormone partially antagonized epinephrine effects on gluconeogenesis and F-2,6-P_2 levels, as well as on pyruvate kinase and PFK-2 activities in hepatocytes isolated from obese rats, without significant changes in the cellular content of cAMP (see Table 1). A defective modulation by insulin of this cAMP-independent pathway (like the impaired activation of one or more protein phosphatases) also could contribute to the resistance of epinephrine-stimulated gluconeogenesis to insulin modulation observed in obese rat hepatocytes.

In summary, in obese *(fa/fa)* Zucker rat hepatocytes, epinephrine-stimulated gluconeogenesis is resistant to short-term modulation by insulin. That impairment in insulin action also affects insulin modulation of epinephrine-mediated effects on pyruvate kinase and PFK-2 activities and on F-2,6-P_2 levels. Our findings further support the idea that hepatocytes isolated from obese *(fa/fa)* rats have an intrinsically defective response to short-term insulin action (13). Under epinephrine stimulation conditions, the observed resistance to insulin action seems to arise, at least in part, from an impairment of the insulin-mediated reduction of hepatocyte cAMP levels in the obese animals.

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